Novel mutation in addition to functional *TMPRSS6* gene polymorphisms originate an IRIDA-like phenotype in an African child

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Introduction

Iron-refractory iron deficiency anemia (**IRIDA**) is a hereditary rare autosomal recessive anemia often unresponsive to oral iron intake and partially responsive to parenteral iron treatment. [1]

The disease originates from mutations in ***TMPRSS6* gene**, encoding **Matriptase-2**, a transmembrane serine protease that plays an essential role in down-regulating hepcidin (Figure 1). [1,2]

Once *TMPRSS6* is mutated, the corresponding protein is absent or inactive at the hepatocyte membrane leading to uncontrolled high levels of hepcidin and impaired iron absorption. [3]

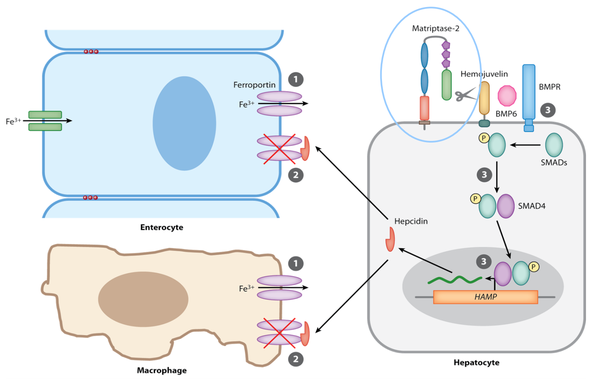


Figure 1. Matriptase-2 (circled in blue) regulates cellular iron export:
(1) Dietary iron and iron recycled from erythrocytes are stored in enterocytes and splenic macrophages and are released to the circulation through ferroportin;
(2) Hepcidin produced by hepatocytes binds to ferroportin and targets the channel for degradation;
(3) Hepcidin gene (*HAMP*) expression is positively regulated by bone morphogenic protein BMP6, which signals through the BMP receptor (BMPR)-SMAD pathway in a hemojuvelin-dependent manner. **Matriptase-2 continuously degrades hemojuvelin, which reduces hepcidin synthesis and allows appropriate ferroportin iron cellular export from enterocytes and macrophages to the bloodstream.**[2]

Aims

This study aimed to investigate a 4-year-old boy of sub-Saharan ancestry (Mozambique/Angola), presenting a microcytic hypochromic anemia, low transferrin saturation, normal ferritin, and having a partial response to intravenous iron treatment (Table 1).

Material and Methods

The subject is a -$\alpha^{3.7}$-thalassemia carrier. *TMPRSS6* was screened for variants by Next-Generation Sequencing using NexteraXT libraries in a *MiSeq* platform (*Illumina*). Genetic variants found were validated by Sanger sequencing. *In silico* analyses were performed in Poly-Phen2, Sorting Intolerant from Tolerant (SIFT) and Missense3D *softwares*.

Table 1: **Hematologic parameters and iron status** of the patient before and after intravenous iron treatment in comparison to reference values.

Hematologic and biochemical parameters	Reference values for 1-4 years old	Patient Iron treatment	
		Before	After
RBC (x10^12/L)	3.50–5.30	4.51	4.38
Hb (g/dL)	10.7–15.1	**9.8**	**10.2**
Ht (%)	31.0–45.0	31.7	33.1
MCV (fL)	72.0–100.0	**70.3**	**75.6**
MCH (pg)	23.8–34.2	**21.7**	**23.3**
MCHC (g/dL)	31.6–34.9	**30.9**	**30.8**
RDW (%)	11.6–13.9	**17.7**	**16.6**
Fe (µg/dL)	62-68	**19**	**51**
TIBC (µg/dL)	228-428	313	273
TSAT (%)	16.0-45.0	**6.07**	18.68
Ft (µg/L)	30.0-300.0	33.0	242.3

Results and Discussion

✓ DNA analysis - **A novel missense mutation** (c.871G>A) was found in heterozygosity, in ***TMPRSS6* exon 8**. Additionally, 3 SNPs previously associated with a greater risk of developing iron deficiency anemia (**K253E, V736A** and **Y739Y**) were also identified in *TMPRSS6* (**Figure 2**). At protein level, the novel variant gives rise to the **G291S** mutation, located at the first CUB1 domain, which suggests it may affect the enzyme activation and substrate recognition (**Figure 2**).

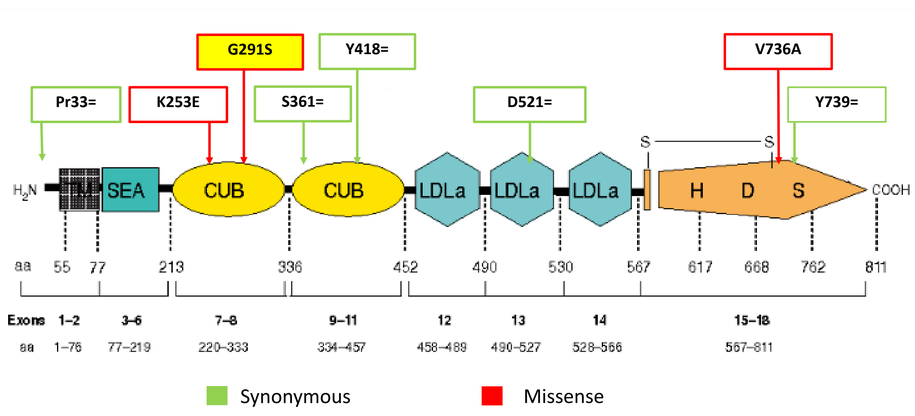
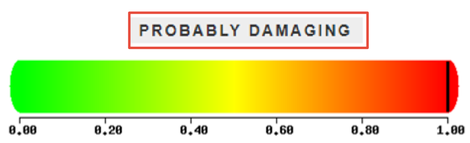


Figure 2: **Schematic model of Matriptase-2.** Structurally, Matriptase-2 contains a short N-terminal cytoplasmic domain, a membrane-spanning region (TM), one SEA domain, two CUB domains, three LDLa domains, and a trypsin-like serine protease domain. Coding region mutations are indicated by arrows. The novel G291S mutation is highlighted in yellow. Adapted from Lee *et al*. 2009 [4]

✓ *In silico* analysis - Indicates the conserved amino acid change (G291S) may be damaging to the protein structure and stability (**Figure 3**).

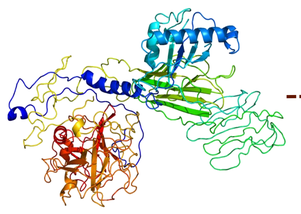
(a)



(b)

dbSNP	# CHR	POS	REF_ ALLELE	ALT_ ALLELE	AA_ CHANGE	SIFT_ SCORE	SIFT_ MEDIAN	#_SEQS_AT_ POSITION	SIFT_ PREDICTION
rs145053404	22	37482452	G	A	G291S	0	3.01	22	DELETERIOUS

(c)



#	Variants	Analysis Results
1	final (-) | 291 GLY > SER	Cavity altered; Gly in a bend

Figure 3. **Results from *in silico* analysis showing potential damage to the protein structure and stability:**
(a) Poly-Phen2, that takes into consideration differences between human proteins and homologous proteins in other mammals (**score = 1; probably damaging**);
(b) SIFT, that predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids (**score = 0; deleterious**);
(c) Missense3D, that perceives the importance of an amino acid based on 3D structure of the whole protein. In order to run the Missense3D analysis, we predicted a 3D structure for Matriptase-2 in the *software* Phyre2iro [**cavity altered; Gly in a bend**].

Conclusions

✓ Although IRIDA is known as an autosomal recessive disease, in this case, the result of a digenic inheritance of the novel damaging mutation (c.871G>A; G291S), three common modulating SNPs in the same gene and the co-inheritance of the α-thalassemia *HBA* deletion may lead to an IRIDA-like phenotype.

✓ Further functional studies of the mutated protein as well as family studies should be conducted.

References

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Acknowledgments

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The authors declare no conflict of interest.